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Faculdade de Medicina Dentária

Ethanol wet bonding: an *in vitro* new approach

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Dissertação

Mestrado Integrado em Medicina Dentária

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“Habe nun, ach! Philosophie,
Juristerei und Medizin,
Und leider auch Theologie
Durchaus studiert, mit heißem Bemühn.
Da steh ich nun, ich armer Tor!”

Goethe

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Abreviates

Bis-GMA	Bisphenol A diglycyl methacrylaye
EWB w/ P	Ethanol wet bonding with primer
EWB w/o P	Ethanol wet bonding without primer
HEMA	Hydroxyethylmethacrylate
MMPs	Matrix metalloproteinases
PF	Premature failures
WWB	Water wet bonding
μTBS	Microtensile bond strength

Resumo:

De acordo com o estado da arte actual, o grande desafio da adesão moderna prende-se com a elevada hidrofilia dos sistemas adesivos contemporâneos. Ao longo da evolução dos sistemas adesivos, a incorporação de monómeros hidrofílicos foi imperativa. No entanto, a incorporação desses monómeros tem efeitos nefastos a longo prazo, conduzindo à degradação hidrolítica e consequente compromisso da durabilidade da adesão dentária. Este problema é mais premente na dentina que no esmalte, pois tem um maior teor orgânico, estabelecendo forças de adesão menos estáveis e previsíveis.

Ao longo dos anos, várias abordagens foram propostas de forma a colmatar os problemas existentes. Durante os anos 90 do século passado, existiu uma grande revolução na abordagem utilizada: a adesão passou a ser feita sobre um substrato húmido, o que implicou uma transição da filosofia de *dry bonding* para *wet bonding*. No entanto, a adesão à dentina continua a não reproduzir os resultados desejados.

No início deste século, ocorreu uma nova mudança no paradigma da adesão e surgiu a filosofia de *ethanol wet bonding*. Esta abordagem ambiciona resolver os problemas relacionados com a elevada hidrofilia dos sistemas adesivos actuais. Desta forma, uma vez que os monómeros hidrofóbicos que compõem os adesivos são solúveis em etanol, foi proposta a aplicação de etanol sobre a dentina recém-condicionada e previamente à aplicação dos restantes componentes do sistema adesivo. Propõe-se que o etanol desidrate a superfície dentária desmineralizada, removendo a água residual e facilitando a infiltração dos monómeros. Na teoria, a as forças adesivas obtidas serão mais estáveis e a durabilidade da adesão será maior.

A literatura apresenta dois protocolos de aplicação do etanol. No primeiro, denominado de técnica progressiva, várias concentrações crescentes de etanol são aplicadas de forma sequencial. Este método apresenta resultados superiores e mais consistentes, no entanto, tem pouca aplicação na prática clínica pois requer muito tempo. O segundo protocolo, denominado de simplificado, preconiza a utilização de etanol na concentração de 100% durante um minuto. Este protocolo apresenta resultados mais variáveis, no entanto, devido à sua simplicidade, parece ser uma opção mais aliciante.

Objectivos: A abordagem ideal desta técnica deve englobar as vantagens dos dois protocolos. O actual estudo preconiza a utilização de um protocolo híbrido, no qual a aplicação de duas concentrações crescentes de etanol é feita durante 60 segundos. Pretende-se obter uma técnica simples, através da utilização de etanol que pode ser

facilmente obtido até no supermercado. Assim, o objectivo deste estudo laboratorial é comparar as forças de adesão à dentina entre o protocolo proposto de *ethanol wet bonding*, variando a aplicação de *primer*, com a técnica convencional de *water-wet bonding*, através de testes de microtração (μTBS).

As hipóteses nulas deste estudo são: (1) o protocolo de adesão utilizado não influencia as forças de adesão obtidas; (2) a aplicação de *primer* não influencia as forças de adesão obtidas; (3) não existem diferenças na distribuição de fracturas entre os grupos estudados.

Materiais e métodos: Uma amostra conveniente composta por quinze molares humanos recentemente extraídos, intactos e sem evidência macroscópica de cárie ou restaurações (n=15), foi distribuída aleatoriamente em três grupos, segundo a estratégia de adesão: Grupo WWB (controlo) – utilização do primer e adesivo do sistema *etch-and-rinse* de 3 passos Adper Scotchbond MultiPurpose (3M ESPE); o Grupo EWB w/ P – aplicação de concentrações crescentes de etanol (70% e 96%) durante 60 segundos, e utilização de um *primer* experimental hidrofóbico, obtido pela diluição do adesivo do sistema adesivo acima descrito em álcool a 96%; e o Grupo EWB w/o P – aplicação de concentrações crescentes de etanol (70% e 96%) durante 60 segundos, sem a utilização de qualquer *primer*. Todos os grupos utilizaram o mesmo adesivo Adper Scotchbond MultiPurpose Adhesive (3M ESPE).

Após a preparação dos dentes e, com o objetivo de formar uma *smear layer* semelhante à que é obtida em situações clínicas, a superfície dos dentes foi polida com papel abrasivo de carbureto de silício (SiC) de grão 600, durante 60 segundos sob refrigeração com água, numa máquina polidora (DAP-U, Struers, Denmark).

Procedeu-se de seguida à aplicação dos protocolos adesivos de acordo com a distribuição nos respetivos grupos experimentais. Em todas as amostras o adesivo foi fotopolimerizado durante 20 segundos com o fotopolimerizador Bluephase® 20i (G2), (Ivoclar Vivadent, Austria) com intensidade de 600 mW/cm^2, controlado periodicamente por um radiómetro (Bluephase® meter, Ivoclar Vivadent, Austria). De seguida foi aplicada a resina composta Herculite™ XRV Ultra™ Dentine, na cor A2, (Kerr Italia S.r.I., Scafati (SA), Italy) em camadas de aproximadamente 2 mm fotopolimerizadas entre si durante 40 segundos. As faces vestibular, lingual, mesial e distal foram polimerizadas adicionalmente por mais 40 segundos cada. A face exterior da resina composta de todos os espécimes foi pintada com tinta à prova de água, com o objetivo de excluir do estudo os palitos nos quais a adesão foi feita em esmalte. Os dentes foram

armazenados em água destilada numa estufa de incubação durante 24h a 37ºC e registado o dia e a hora da reconstrução.

Posteriormente, foram efetuados cortes no eixo do "x" e do "y" com um disco de diamante, a baixa rotação e sob refrigeração com água, num micrótomo de tecidos duros (IsoMet^{TM} Diamond Wafering Blade -10,2cmx0,3mm - Series 15HC, Buehler Ltd., Lake Bluff, IL, USA), com o objetivo de obter palitos com uma área de, aproximadamente, 0,7 mm^2. Cada palito foi colado individualmente num suporte de Geraldelli´s, com cola de cianoacrilato, e testados um a um. Foram sujeitos a forças de tração numa máquina de Teste Universal, a uma velocidade de 1 mm/minuto até ocorrer fratura. Mediu-se a secção de cada espécimen fraturado com uma craveira digital e determinou-se a área em milímetros quadrados (mm^2). A área de superfície de cada palito e a sua resistência à fratura, medida em KiloNewtons (KN), foram registadas e, a partir delas, calculadas as forças de adesão em MegaPascais (MPa). Cada fratura foi observada ao estereomicroscópio (Nikon, Japan) com uma ampliação de 10x, para se caracterizar o tipo de fratura ocorrida (coesiva, adesiva ou mista). Quando a fratura ocorreu exclusivamente na dentina foi denominada como coesiva de dentina (CD) e quando ocorreu exclusivamente na resina composta foi classificada como coesiva de compósito (CC). Quando a fratura ocorreu na interface dentina-adesivo, denominou-se de adesiva (A) e, quando atingiu tanto a dentina como a resina composta, foi denominada como mista (M).

A análise estatística dos resultados foi realizada através do teste paramétrico ANOVA, após se ter verificado que a amostra seguia uma distribuição normal (os testes de Kolmogorov-Smirnov e Shapiro-Wilk foram usados para avaliar se os resultados seguiam uma distribuição normal; o teste de Levene foi usado para determinar a igualdade de variâncias). O intervalo de confiança definido foi de 95%. O número de palitos fraturados durante a sua preparação (palitos descolados) foi registado e os valores foram considerados para a análise estatística

Resultados: Foram testados 239 palitos, 111 correspondentes ao grupo WWB, 125 correspondentes ao grupo EWB w/P e 3 correspondentes ao grupo EWB w/o P. As forças de adesão quando a técnica de EWB w/P foi aplicada (27,1868 ± 11,91210 MPa) foram superiores às forças de adesão quando do grupo de WWB (25,6570 ± 5,36309 MPa). Ambos os grupos obtiveram forças de adesão superiores às obtidas pelo protocolo de EWB w/o P (2,4998 ± 0,34510 MPa). A análise estatística com ANOVA determinou a existência de diferenças estatisticamente significativas entre estes grupos (p=0,000). Análise estatística com o teste de Tukey permitiu apurar que existem diferenças

estatisticamente significativas entre quando se comprara o grupo de EWB w/o P com os outros dois grupos (p=0,001) e que não existem diferenças estatisticamente significativas entre os grupos de WWB e EWB w/ P (p=0,945). Após a observação do tipo de fratura ocorrida, verificou-se que, no total dos três grupos, 87,6% foram fraturas adesivas, 4,4% mistas, 6,2% coesivas de compósito e 2,7% coesivas de dentina.

Conclusões: Tendo em conta as limitações deste estudo laboratorial, pode-se concluir que a técnica de EWB w/P apresenta resultados semelhantes aos obtidos pela técnica de WWB. No entanto, a técnica de EWB w/o P apresenta resultados inferiores aos outros dois grupos. Conclui-se também que não existem diferenças relativamente à distribuição de fracturas em cada um dos grupos Estudos futuros poderão avaliar o efeito a longo prazo do armazenamento em água no desempenho deste protocolo. Além disso, estudos em diferentes substratos, como por exemplo, em dentina terciária, após remoção da lesão de cárie. Bem como estudos de nanoinfiltração, em associação com estudos *in vivo* são necessários para avaliar o desempenho clínico desta nova classe de adesivos, para que possam ser utilizados futuramente com maior conhecimento.

***Palavras-chave*:** adesão à dentina, *ethanol-wet bonding*, forças de adesão à microtracção.

Abstract:

Objetives: The purpose of the present study is to evaluate and compare the immediate resin-dentin bond strength produced by WWB and by an experimental approach of the EWB technique, using microtensile bond strength (μTBS); and to clarify the influence of an experimental primer on the *in vitro* performance of EWB approach proposed in the present study. The null hypotheses tested were: (1) bonding protocol had no effect on the bond strength; (2) primer application had no effect on the bond strength; (3) there are no differences in the distribution of fractures across all tested groups.

Methods: Fifteen recently extracted human molars, intact and without macroscopic evidence of caries or restorations, were assigned to three groups according to the etching strategy: Group WWB (control) – Adper Scotchbond Multipurpose Primer and Adhesive (3M ESPE) applied as a 3-step etch-and-rinse adhesive on moist dentin; Group EWB w/P – experimental series of increasing ethanol concentrations (70% and 96% during 60 seconds)applied, followed by an experimental hydrophobic primer, formulated by diluting 50 wt% Adper Scotchbond Multipurpose Adhesive (3M ESPE) in 96% ethanol; and EWB w/o P – experimental series of increasing ethanol concentrations (70% and 96% during 60 seconds) applied without any primer. The same adhesive was applied in all groups: Adper Scotchbond MultiPurpose (3M ESPE).After resin composite build-ups were performed, the teeth were stored in distilled water in an incubator for 24 hours at 37°C. Specimens were sectioned with a slow-speed diamond saw under water irrigation to obtain bonded sticks that were tested to failure in a universal testing machine at a crosshead speed of 1mm/minute. The statistical analysis of the results was performed with SPSS. A one-way ANOVA test was performed when the assumption of normality was valid.

Results: The mean μTBS to dentin of EWB w/ P was statistically similar to WWB (p=0,001). The mean μTBS to dentin of EWB w/o P was statistically lower than both EWB w/P and WWB (p=0,945). Most fractures were adhesives (87,6%)

Conclusions: Within the limitations of the present laboratory study EWB w/ P showed similar bonding effectiveness to WWB, after 24 hours. EWB w/o P showed lower bonding effectiveness when compared to the other two groups. There are no differences in the distribution of fractures across all tested groups.

Keywords: Dentin bonding, ethanol-wet bonding, microtensile bond strengths.

Introduction:

Throughout the years, the revolution in modern dentistry has provided the means to accomplish a more conservative approach, leading to increasingly aesthetic treatments. The remarkable advances of dentin-bonding technology made possible the use of composites, a tooth-colored resin-bonded restorative material widely used in clinical practice. The clinical outcome of bonded restorations is intrinsically dependent of bonding to dentin, making improvements in this field a subject of continuing interest (Huang *et al*., 2011; Liu *et al*., 2011; Khoroushi *et al*, 2014).

Despite all improvements in adhesive systems, the longevity of the restorations is still a problematic issue. Dentin-bonding represents a challenge due to the high content of water and organic components in dentin, making it less stable and predictable than enamel-bonding (Breschi *et al*., 2008; Perdigão *et al.*, 2013; Ayar *et al*., 2014; Khoroushi *et al*, 2014; Yesilyurt *et al*. 2015; Ayar, 2016).

Even though the immediate bond strength values of current adhesives have been shown to be quite high, aging leads to a significant decrease in resin-dentin bond strength. The hybrid layer at the adhesive interface degrades over time, weakening adhesion and ultimately leading to the loss of the bonded restoration. Hence, efforts have been made to extend the clinical lifetime of bonded restorations, focusing on enhancing the stability of the bond to tooth issue (Tay *et al*., 2007; Breschi *et al*., 2008; Liu *et al*., 2011).

With the aim of increasing the lifespan of the resin-dentin bond, the water wet bonding (WWB) technique was introduced in the early 1990's. This technique seemed a promising way to prevent the collapse of the demineralized dentin collagen fibrils after acid etching, since the etched dentin is kept moist, increasing the penetration of the resin into the etched tooth surface. (Liu *et al*., 2011; Spencer *et al*., 2011; Mortazavi *et al*., 2012; Perdigão *et al.*, 2013).

The presence of water in the etched dentin imposed a new bonding strategy and manufacturers had to develop new adhesive systems, since the main component of these systems, the bisphenol A diglycyl methacrylaye (Bis-GMA) monomer, has limited water solubility. To avoid this problem, manufacturers incorporated hydrophilic monomers, such as hydroxyethylmethacrylate (HEMA), into dentin adhesives. HEMA acted as a solvent for non-water-compatible resin monomers, enhancing wettability and reducing

the phase separation, making these resins more compatible with moisty acid-etch dentin (Van Landuyt *et al*.; 2007; Liu *et al*.; 2011; Perdigão *et al.*, 2013).

Even though water plays a key role in enhancing the early stage of resin infiltration, it also promotes degradation of the resin interface. Improving the hydrophilic nature of these systems has several drawbacks, including the increased water adsorption after polymerization, which leads to plasticization of the adhesives and lowers their mechanical properties (Ito *et al.*, 2005; Van Landuyt *et al*., 2007; Kostoryz *et al*., 2009; Liu *et al*., 2011; Spencer *et al*.; 2014; Tjäderhane, 2015).

Besides hydrolysis caused by water sorption, the durability of resin-dentin bond is affected by the residual water. Despite all advances, contemporary adhesives cannot replace free and unbound water from the interfibrillar spaces, creating water-filled channels within hybrid layers and leading to insufficient penetration of resin into the collagen fibrils. The exposed collagen fibrils, along with collagen fibrils poorly enveloped by resin, are vulnerable to slowly degradation by collagenolytic enzymes such as matrix metalloproteinases (MMPs) (De Munck *et al*., 2009; Kostoryz *et al*., 2009; Osorio *et al*., 2010; Kim *et al*., 2010; Liu *et al*., 2011; Pashley *et al*., 2011; Bertassoni *et al.*, 2012).

Thus, all these factors contribute to a decrease of the resin-dentin bond strength over time, accelerating degradation of the resin-adhesive interface and leading to loss of restoration (De Munck *et al*., 2003; De Munck *et al*., 2004; Mazzoni *et al*., 2007; Vaidyanathan & Vaidyanathan, 2009; Pashley *et al*., 2011; Grégoire *et al.*, 2013; Tjäderhane *et al.*, 2013).

To overcome this issue, it has been proposed that future dentin adhesives should be rendered less hydrophilic and efforts have been made to find a technique that optimize the infiltration of hydrophobic monomers into the wet demineralized dentin and solve the problems associated with contemporary adhesive systems (Bertassoni *et al.*, 2012; Mortazavi *et al*., 2012; Pei *et al*., 2012; Araújo *et al*., 2013; Souza Júnior, 2015).

In recent years, a paradigm shift led to the development of a new bonding philosophy known as ethanol-wet bonding (EWB). Firstly introduced by Pashley *et al.*, 2007 as an experimental strategy, it relies on the idea that water replacement from interfibrillar and intrafibrillar spaces by ethanol through a dehydration/saturation process, provides a fairly hydrophobic, ethanol-suspended demineralized collagen matrix for

infiltration by resin monomers (Nishitani *et al*., 2006; Pashley *et al.*, 2007; Osorio *et al*. 2010; Sadek *et al*.; 2010a). EWB embodies a major impact in adhesive dentistry, since the philosophy behind it reveals the critical barrier to progress in dentin bonding with etch-and-rinse and self-etch adhesives (Liu *et al*., 2011; Tjäderhane *et al*. 2013).

The concept of EWB may be explained in terms of solubility parameter theory. The rationale behind the use of ethanol is that miscibility of both hydrophobic and hydrophilic monomers in the ethanol-saturated dentin is better than those in the water-saturated dentin. These monomers are components in most of the dental adhesives currently available (Sadek *et al*., 2008; Hosaka *et al*., 2009; Sadek *et al*., 2010a; Ayar, 2016).

According to EWB concept, ethanol is applied prior to primer and adhesive, representing an extra step in bonding. Furthermore, this technique creates hybrid layers containing collagen fibrils with reduced fibrillar diameter and wider interfibrillar spaces, allowing better infiltration of hydrophobic resin and collagen encapsulation. Ethanol wet bonding also creates bonded interfaces with reduced micropermeability and forms a more hydrophobic hybrid layer. The obtained hybrid layer shows decreased water sorption and resin plasticization and increased resistance to cleavage of collagen, avoiding phase separation. Thereby, this prevents hybrid layer degradation, extending the longevity of resin-dentin bonds (Hosaka *et al*., 2009; Liu *et al*., 2011; Sauro *et al*., 2011; Tjäderhane, 2015).

Consequently, several laboratory studies have demonstrated that ethanol wet bonding technique results in bond strength values equal or higher than those produced by conventional adhesive techniques (Nishitani *et al*., 2006; Sadek *et al.*, 2008; Hosaka *et al.*, 2009; Huang *et al*., 2011; Araújo *et al.*, 2013; Ayar, 2016).

Being a relatively new concept, there seems to be some disparity regarding to how ethanol application should be performed. The literature shows a myriad of protocols with great variability in terms of applied concentrations, number and time of applications. However, there are primarily two main protocols. (Sadek *et al.*, 2008; Ayar, 2014).

The first one, known as full-dehydration protocol, comprises the application of series of increasing ethanol concentrations (50%, 70%, 80%, 95% and 100% ethanol three times for 30 seconds each). This progressive technique provides a gradual water replacement and avoids collapse of the interfibrillar spaces within the collagen matrix. It

should be stated that these ethanol concentrations are not easily available, which represents an evident disadvantage. Despite showing higher consistency of results, this approach is complex and time-consuming, becoming clinically impracticable (Pashley *et al.*, 2007; Sadek *et al*., 2008; Ayar, 2014; Yesilyurt *et al.*, 2015; Ayar 2016).

A second protocol, known as simplified technique, advocates the application of 100% ethanol concentration only once, for 60 seconds. Even though this user-friendly technique shows the potential for use in clinical practice, it is extremely technique sensitive. Special care should be taken to prevent the collapse of the collagen matrix caused by water evaporation during the transition from the water to the ethanol phase, as it can result in stiffening and stabilization of the matrix in its collapsed state. It has been shown that simplified technique is not able to adequately replace water within dentin, yielding lower bond strengths (Sadek *et al*., 2010b; Sadek *et al*., 2010c; Sauro *et al*., 2011; Guimarães *et al*., 2012; Li *et al*., 2012; Ayar, 2014).

For both techniques, it is imperative that ethanol application is meticulously performed. When water-saturated collagen is exposure to air, the surface tension present along the collagen interface can collapse the collagen matrix, inhibiting optimal infiltration of the adhesive monomers. Thus, after ethanol dehydration, adhesives should be readily applied in the ethanol-wet dentine to avoid the collapse of the collagen network (Sadek *et al.*, 2010b; Sadek *et al*., 2010c; Huang *et al.*, 2011; Liu *et al*. 2011).

It can be concluded that an ideal approach should embrace the advantages of both protocols. The present study advocates a hybrid protocol, which comprises the use of ascending ethanol concentrations (70% and 96% only once, for 30 seconds each) during 60 seconds. These two ethanol concentrations are easily available at a supermarket, which fulfils the principle of user-friendly dentistry.

Microtensile bond strength test

As previously stated, despite the rapid evolution of dental adhesive technology, the durability of the adhesive interface remains the Achilles heel of an adhesive restoration. The bedrock to avoid structural failure is that the stress applied must not

exceed the strength of the material. For dentin adhesion, this implies that the bonding failure can be avoided if the bond strength of resin to dentin is superior to the stress applied to a restoration (Van Meerbeek *et al.*, 2010; Spencer *et al.*, 2011).

In order to predict the performance of the bonding interface, diverse methodologies can be used. Clinical trials (*in vivo* studies) remain the ultimate method to assemble scientific evidence on the clinical effectiveness of a restorative procedure. Nevertheless, clinical trials are highly complex and their outcome depends upon diverse factors, such as patient compliance and the number of patients required (Perdigão & Lopes, 1999; Van Meerbeek *et al*., 2003).

Therefore, laboratory tests (*in vitro* studies) are used to predict the eventual clinical outcome. These tests can gather data on a specific parameter and evaluate the effect of a single variable, while keeping all other variables constant, using relatively unsophisticated and inexpensive test protocols and instruments (Swift *et al*., 1995).

Several methodologies can be used to measure the bonding effectiveness of adhesives to enamel and dentin. Bond strength tests are the most frequently used. The rationale behind this testing method is that the stronger the adhesion between tooth and biomaterial, the better it will resist stress imposed by resin polymerization and oral function. The bond strength can be measured statically (macro-shear, macro-tensile, micro-shear, micro-tensile) or dynamically (fatigue test) (Van Meerbeek *et al*., 2010).

In 1994, Sano *et al*. introduced the microtensile bond strength (μTBS) test. This methodology has been recognized as a versatile and reliable *in vitro* static test to quantify the bonding effectiveness and stability of adhesive biomaterials bonded to tooth structure. It appears to be able to discriminate adhesives better on their bonding performance than a traditional shear bond strength approach, being the most employed test in current scientific papers reporting on bond strengths (Pashley *et al*., 1995; Pashley *et al*., 1999; Van Meerbeek *et al*., 2010).

A long list of advantages is attributed to μTBS. It is an economic test (a single tooth origins multiple micro-specimens), with the ability to measure regional bond strengths (e.g. peripheral *versus* central dentin) and allows testing of both small areas and bonds to irregular surfaces. Pashley *et al*. (1995) stated that this test has more adhesive failures and fewer cohesive failures and allows the measure of higher interfacial bond. It also enables the calculation of means and variances for single teeth and examination of

the failed bonds by scanning electron microscopy, since the surface area is approximately 1 mm^2.

Yet, some disadvantages of μTBS test have been reported. It is a laborious and technically demanding test and requires special equipment. Bond strengths lower than 5 MPa are not easily measured and this test requires samples so small that they dehydrate and damaged easily (Pashley *et al*., 1995; Pashley *et al*., 1999).

There is little information in the literature about the performance of EWB *(Li et al,*. 2012). While some *in vitro* studies have shown that EWB did not affect the bond strength, other studies demonstrated that bond strengths of both of hydrophilic and hydrophobic adhesive systems have been improved when this technique was used (Osorio *et al*. 2010).

Objectives:

Experimental *in vitro* study with the aim to evaluate and compare the immediate resin-dentin bond strength produced by WWB and by an experimental approach of the EWB technique, using microtensile bond strength (μTBS); and to clarify the influence of an experimental primer on the *in vitro* performance of EWB approach proposed in the present study, according to the following null hypothesis.

- Bonding protocol had no effect on the bond strength.
- Primer application had no effect on the bond strength.
- There are no differences in the distribution of fractures across all tested groups.

Materials and methods:

1. Design of the study

A convenient sample of fifteen recently extracted human molars, intact and without macroscopic evidence of caries or restorations, was used on this study. Before their preparation, the teeth were randomly selected from a group of teeth firstly stored in 0,5% Chloramine T (Sigma Chemical Co., St Louis, MO, USA) at 4ºC for one week, according to the ISO/TS 11405 standard (ISO/TS 11405:2003) and then, left in distilled water at 4º C according to the ISO/TS 11405 standard (ISO/TS 11405:2003), no more than 6 months.

All teeth were cleaned under running water and all adherent tissues were removed using a periodontal scaler.

2. Teeth preparation

The teeth crowns were attached to an acrylic holder with sticky wax, perpendicular to the long axis of the tooth. Under constant distilled water irrigation and using a precision diamond disk at low speed (IsoMet™ Diamond Wafering Blade - 10,2cmx0,3mm - Series 15HC, Buehler Ltd., Lake Bluff, IL, USA) on a hard tissue microtome (IsoMet® 1000 Precision Saw, Buehler Ltd. Ltd., Lake Buff, IL, USA), two cuts were made. The first cut was made parallel to the occlusal surface, 1-2 mm below the cementoenamel junction, to remove the roots and expose the pulp chamber (Figure 1). Then, the crowns were detached from the acrylic holders and the pulp tissues were removed from the pulp chamber with a dentin curette (Figure 2). The pulp chamber was then filled with cyanoacrylate glue (Loctite Super Cola 3 Precisão, Henkel, Germany) and the crowns were fixed with the same glue to the acrylic holders, by the sectioning surface.

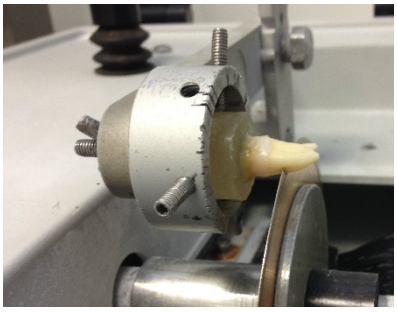

Figure 1 *– Tooth attached to an acrylic holder with sticky wax. Root cutting 1-2 mm below the CEJ.*

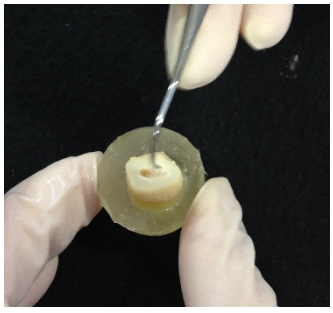

Figure 2 *– Removal of the pulp tissues.*

A second cut was made parallel to the first one, in order to obtain mid-coronal dentin surfaces. This second cut removed both the occlusal enamel and superficial dentin of the molar crowns (Figure 3).

In order to create a uniform smear layer obtained in similar conditions of those occurring in clinic situations, the dentin surface was polished with 600-grit silica-carbide (SiC) sandpaper (CarbiMet^{TM} SiC Abrasive Disk 600 [P1200] – 20,0mm – Buehler Ltd., Lake Bluff, IL, USA), for 60 seconds under water irrigation, on a mechanical grinder (DAP-U, Struers, Denmark), according to the ISO/TS 11405 standard (ISO/TS 11405:2003) (Figure 4).

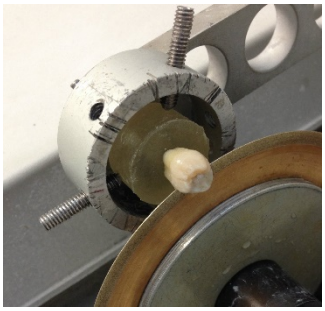

Figure 3 *– Removal of the occlusal enamel and superficial dentin.*

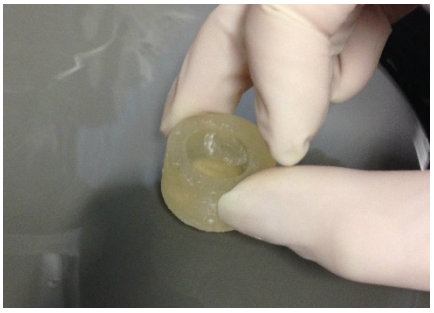

Figure 4 *– Creating the smear layer on a mechanical grinder.*

3. Distribution and treatment of the crown segments

The fifteen crown segments were randomly assigned to one of the three groups (n=5), according to the different dentin conditioning methods (Table 1). The order in which the crown segments were treated was random, to avoid possible bias due to any particular sequence of treatment. All the treatment procedures were performed by the same operator.

Table 1- Testing groups

	Testing groups (n=5)		
	***Conventional Water-wet bonding* (control group)**	***Simplified ethanol-wet bonding with primer***	***Simplified ethanol-wet bonding without primer***
Microtensile bond strength (μTBS)	WWB (Group 1)	EWB w/ P (Group 2)	EWB w/o P (Group 3)

In all groups, the dentin surfaces of the crown segments were etched for 15 seconds with a 37,5% phosphoric acid gel (Kerr Italia S.r.I., Scafati (SA), Italy). After acid etching, the surface was rinsed with water for 15 seconds (Figure 5). The excess of water was removed from the dentin surface using a moist cotton pellet so that the surface remained shiny and visibly moist, to prevent collapse of the collagen matrix.

In Group A, the control group, the water-wet bonding technique was performed using Adper Scotchbond Multi-Purpose Primer and Adhesive (3M ESPE, St. Paul, MN, USA). The primer was applied for 30 seconds to tooth surface with a disposable microbrush. The surface was then gently air-dried for 5 seconds, until it ceases to show any movement and the solvent was evaporated completely, forming a homogenous and slightly shiny film. If the dentin surface was overdried and didn't remain visibly moist, a second coat of primer was applied.

For Groups B and C, an experimental simplified ethanol-wet bonding protocol was used. In both groups, after acid-etching, the dentin surface was treated with an experimental series of increasing ethanol concentrations: 70% and 96% (Continente, Portugal) ethanol applications, following a chemical dehydration protocol (Figure 6). Each concentration was applied by gently scrubbing for 30 seconds, using a disposable microbrush, giving a total application time of 60 seconds. Special attention was taken to ensure that the dentin surface was always visibly moist prior to the application of the subsequent higher ethanol concentration. After the ethanol application, excess ethanol was removed by gentle blotting with filter paper, leaving the dentin surface visibly moist.

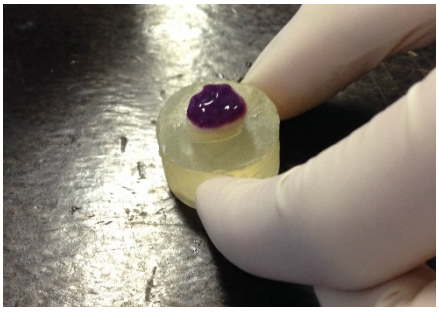

Figure 5 *– Etching the dentin surface.*

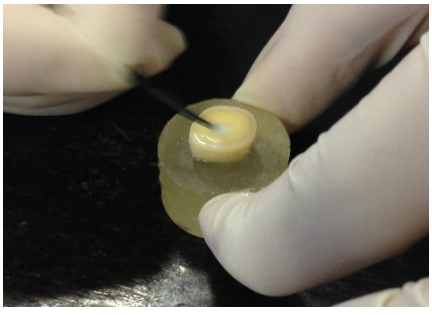

Figure 6 *– Vigorous ethanol application.*

In Group B, after the chemical dehydration with ethanol that was previously described, an experimental primer was used. This experimental hydrophobic primer, formulated by combining 50 wt% resin monomers mixtures with 50 wt% ethanol was obtained by diluting 2 mL of Adper Scotchbond Multipurpose Adhesive (3M ESPE, Neuss, Germany) in 96% ethanol. This primer was applied as described in Group A (Figure 7).

In Group C, no primer was applied after the chemical dehydration protocol.

Then, in all groups, the adhesive (Adper Scotchbond Multipurpose Adhesive, 3M ESPE, Neuss, Germany) was applied to the entire dentin surface, using a disposable microbrush, uniformly creating a thin coating. The adhesive excess was removed by gently air-drying. Finally, the surface was polymerized for 20 seconds (Figure 8).

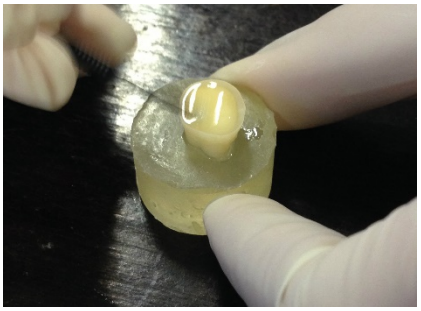

Figure 7 *– Experimental hydrophobic primer being applied.*

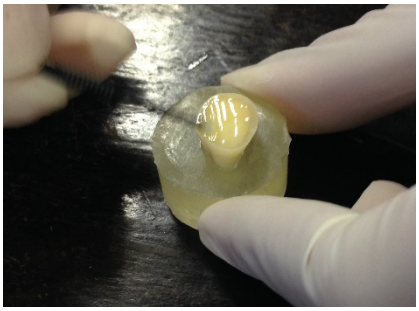

Figure 8 *– Adhesive being applied.*

Resin composite build-ups were performed using a resin composite, Herculite™ XRV Ultra™ Dentine (Kerr Italia S.r.I., Scafati (SA), Italy), shade A2, in three increments of 2 mm each. Each increment was light cured for 40 seconds, according to the manufacturer's instructions, until reaching a height of 6 mm. Additional light polymerization was performed on facial, lingual, mesial and distal surfaces.

All light curing was performed with a light intensity of 600 mW/cm^2, using a LED polymerization unit (Bluephase® 20i (G2), Ivoclar Vivadent, Austria) held 1-2 mm from the treatment surface The output of the curing light was periodically verified throughout the procedure, using a radiometer (Bluephase® meter, Ivoclar Vivadent, Austria).

4. Specimens preparation for micro-tensile tests

After restorative procedures, all teeth were painted using different colors with waterproof ink. The exterior surface of the resin composite was painted in order to identify, and then, exclude from the study, the sticks in which the adhesion was made to enamel (Figure 9).

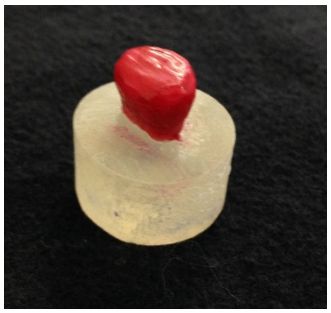

Figure 9 *– Resin application build up, after being painted with waterproof ink.*

Then, the specimens were used to evaluate the microtensile bond strength 24 hours after the restorative procedures (short-term test). The specimens were stored in distilled water in an incubator (TK/L 4105, EHRET GmbH & CO. KG, Germany) for 24 hours at 37º C according to the ISO/TS 11405 (ISO/TS 11405:2003) standard. Date and time of the restoration was registered.

Subsequently, after storage, the teeth were longitudinally sectioned in both "x" and "y" directions with a low-speed diamond disk (IsoMet™ Diamond Wafering Blade - 10,2cmx0,3mm - Series 15HC, Buehler Ltd., Lake Bluff, IL, USA)), under water irrigation, using a hard tissue microtome (IsoMet® 1000 Precision Saw, Buehler Ltd. Ltd., Lake Buff, IL, USA), to obtain bonded sticks with a cross-sectional area of approximately 1 mm^2. Firstly, cuts were spaced approximately 1 mm apart and oriented parallel to the long axis of the tooth ("x" direction) (Figure 10). Then, the tooth was then rotated 90 degrees and other cuts, spaced as described before, were made ("y" direction) (Figure 11).

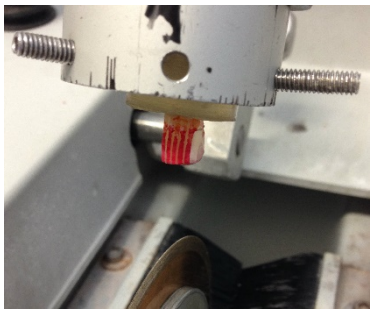

Figure 10 *– Specimen being cut in the "x" direction.*

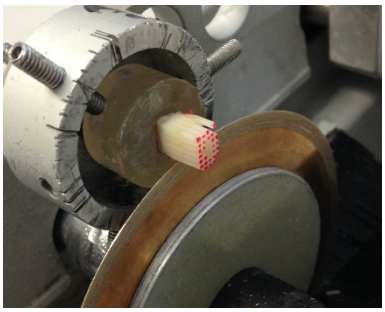

Figure 11 *– Specimen being cut in the "y" direction.*

A final cut was made at the base of the crown, perpendicular to the long axis of the tooth, to separate the sticks from the acrylic holders (Figure 12).

Debonded or lost sticks were registered. Debonded sticks were those separated in the adhesive interface during the cutting procedure. Lost sticks where those which were lost or fractured during test preparation.

The obtained sticks were immediately subjected to microtensile tests.

Figure 12 *- Sticks obtained after the final cut.*

5. Microtensile tests

The sticks were individually attached to a stainless-steel grooved Geraldelli's jig with cyanoacrylate glue (737 Black Magic Toughened adhesive, Permabond, Hampshire, UK) (Figure 13) and then submitted, one by one, to a tension load using a universal testing machine (Instron 4502 Series, Serial no. H3307, Instron Corporation, Canton, MA, USA), at a crosshead speed of 1 mm/min, until fractured occurred (Figure 14).

After fracture, each stick was removed from the testing apparatus and a digital caliper (Absolute Digimatic Caliper, Mitutoyo Corporation, Japan) was used to measure the sides of the bonding interface, given by the cross sectioned area at the site of fracture, and calculate the bonding area in mm^2 of each fractured stick. Both the load at fracture

(kN) and the bonding surface area of the specimens were registered. Then, the μTBS values (μ-tensile bond strength) were calculated in MPa, by dividing the load of fracture by the bonding surface area.

Figure 13 *– The specimen attached to a Geraldelli's jig.*

Figure 14 *– Instron 4502, universal testing machine.*

The sticks that failed prematurely during specimen preparation were registered as the average value between zero and the lowest bond strength value obtained in all experiment (Luque-Martinez *et al*., 2014).

Failures were analyzed by the same observer, under a stereomicroscope (Nikon, Japan) at 10x magnification to determine the failure mode. The failure modes were classified as: 1) cohesive in dentin (CD), when the failure occurred in dentin; 2) cohesive in composite (CC), when the failure occurred in composite; 3) adhesive (A) when failure occurred at the dentin-adhesive interface; 4) mixed (M) when the failure involved both composite and dentin at the interface

Statistical analysis

The statistical analysis of the results was performed through descriptive and inference methods, using the software program SPSS Statistics for MAC Version 21.0 (SPSS Inc., Chicago, IL, USA).

Before submitting the data to the appropriate statistical analysis, the Shapiro-Wilk Test was performed to assess whether the data followed a normal distribution and the Levene's test was computed to determine if the assumption of equality of variances (homoscedasticity) was valid (Table 2).

Since the normality of the data distribution and the equality of the variances were observed in two groups (p>0,05), the microtensile bond strength data was subjected to an one-way analysis of variance (ANOVA) and a *post-hoc* test (Tukey's test) was used for pairwise comparisons. Significance was set at a 95% confidence level.

The number of prematurely debonded specimens (pretesting failures that occurred during specimen preparation) was recorded and included in the statistical analysis. As previously stated, the average value attributed to specimens with premature failures (PF) during preparation corresponded to the value between zero and the lowest bond strength value obtained in all experiment. In this specific study, the value of 1,914351 MPa was attributed when PF were recorded (Luque-Martinez *et al.*, 2014).

Table 2 *- Test for Equality of Variances of the µTBS in MPa*

Levene Statistic	df1	df2	Sig.
3,533	2	12	0,062

Results:

Microtensile bond strength

The mean values in MPa and standard deviations (SD) of the microtensile bond strength for each group are listed in Table 3 and shown in Figure 15. The number of specimens (N), minimum (Min), maximum (Max) are also summarized.

Table 3 - *Descriptive statistics of the µTBS in MPa for the three experimental groups tested. Mean values that are not significantly different from one another share the same upper case letter at p<0,05.*

Group	N	Mean ± SD	Min	Max
WWB	5	25,6570 ± 5,36309 ^{A}	19,50	34,13
EWB w/ P	5	27,1868 ± 11,91210 ^{A}	17,09	47,25
EWB w/o P	5	2,4998 ± 0,34510 ^{B}	2,29	3,09
Total	15	18,4479 ± 13,61861	2,29	47,25

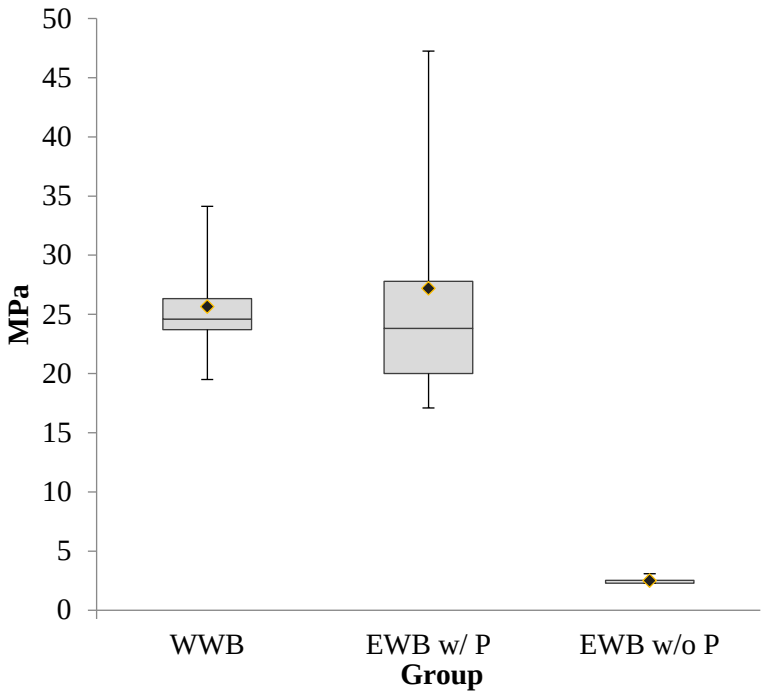

Figure 15 *- Box-whisker plots of the µTBS in MPa for the two different experimental groups tested. The median µTBS is represented by the central line. The box represents the interquartile range. The mean µTBS is represented by the diamond mark (♦).*

The highest mean μTBS was obtained when bonding was performed using EWB with primer (27,1868 ± 5,32725), followed by WWB (25,6570 ± 2,39845). The lowest was obtained when bonding was done using EWB without primer (2,4998 ± 0,15433) (Figure 16).

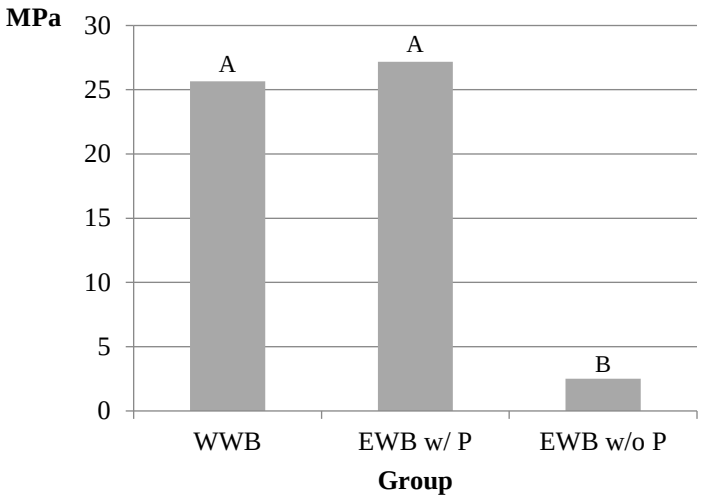

Figure 16 – *Mean μTBS value for each group. Mean values that are not significantly different from one another share the same upper case letter at p<0,05.*

After verifying the normality of the data distribution and the equality of the variances, data from μTBS were analyzed using one-way ANOVA, which revealed that there was a significant difference among groups (p = 0,000) (Table 4).

Table 4 *– One-way ANOVA test*

	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	1913,411	2	956,706	16,806	0,00
Within Groups	683,120	12	56,927		
Total	2596,531	14			

There is much difference between the two Mean Squares (956,706 and 56,927), resulting in a significant difference (F = 16,806; Sig. = 0,000). This means that the null hypothesis must be rejected. Tukey's HSD test was applied to statistically evaluate the

difference in the mean bond strength of the experimental groups (Table 5). The significance level was set at α = 0,05 for all tests.

Table 5– *Tukey HSD Post Hoc Test (α=0,05)*				
(I) Group	**(J) Group**	**Mean Difference (I-J)**	**SE**	**Sig.**
WWB	EWB w/ P	-1,52980	4,77186	0,945
	EWB w/o P	23,15723	4,77186	0,001
EWB w/ P	WWB	1,52980	4,77186	0,945
	EWB w/o P	24,68703	4,77186	0,001
EWB w/o P	WWB	-23,15723	4,77186	0,001
	EWB w/ P	-24,68703	4,77186	0,001

Table 6 *– Tukey HSD test. Mean μTBS values in MPa for groups in homogeneous subsets are displayed.*			
		Subsets for α=0,05	
Group	**N**	**1**	**2**
EWB w/o P	5	2,4998	
WWB	5		25,6570
EWB w/ P	5		27,1868
Sig.		1,000	0,945

Tukey's test revealed that mean μTBS values obtained with EWB without primer were significantly lower than those obtained with approaches using both WWB and EWB with primer (*p*=0,001). There are no statistically significant differences among the two other groups (WWB and EWB with primer) (p=0,945) (Table 5 and 6). Table 6 represents, in a more intuitive way, which groups are statistically similar, since they were listed in the same subset.

Failure mode distribution

Failure mode distribution of the debonded specimens per tested group is shown in Table 7 and 8, Figures 17 and 18. Four failure patterns were depicted: adhesive (A), mixed (M), cohesive in dentin (CD) and cohesive in composite (CC).

Fracture analysis revealed that failure pattern was predominantly adhesive in all groups tested (86,7%). Mixed failures were observed in 4,4% of the specimens. Cohesive failures in composite and dentin were observed in 6,2% and 2,7% of the specimens, respectively.

Table 7 *- Number of specimens according to the failure mode and premature failures of the three different experimental groups tested.*

		Mode of failure					Pretesting failures
		A	M	CC	CD	Total	
Group	**WWB (1)**	100	14	18	9	141	29
	EWB w/ P (2)	131	6	10	3	150	25
	EWB w/o P (3)	159	0	0	0	159	156
Total		390	20	28	12	450	210

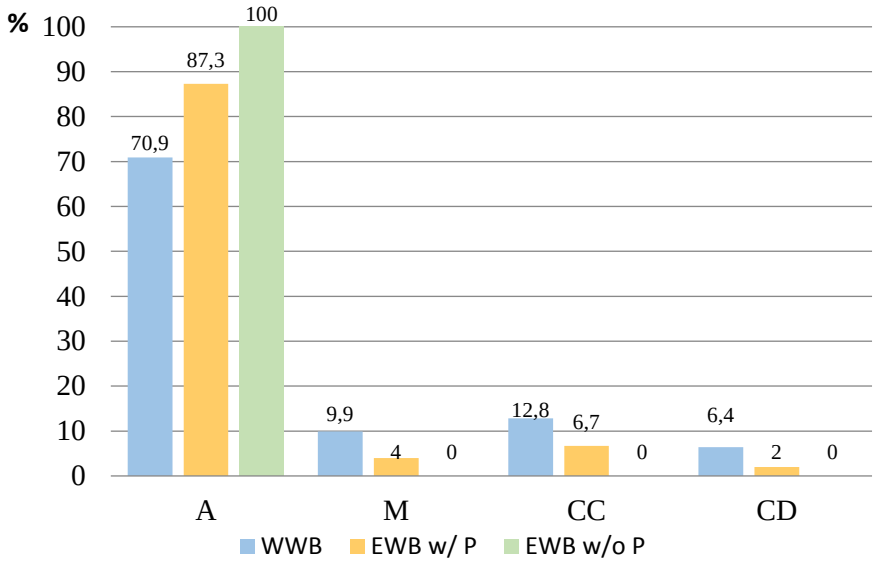

Figure 17 *- Distribution of the specimens according to the failure mode of the three different experimental groups tested.*

Table 8 *- Number of specimens according to the failure mode and percentages of all experimental groups tested.*		
Mode of Failure	**N**	**%**
Adhesive (A)	390	86,7
Mixed (M)	20	4,4
Cohesive in composite (CC)	28	6,2
Cohesive in dentin (CD)	12	2,7
	450	100

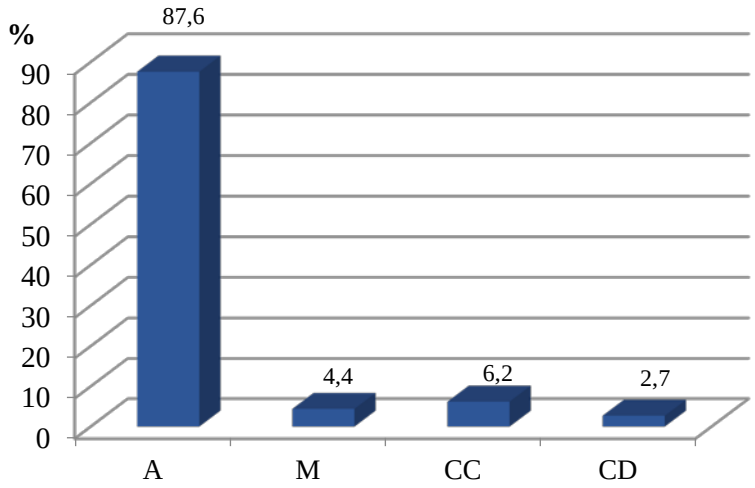

Figure 18*- Distribution of the specimens according to the failure mode of all experimental groups tested.*

Discussion:

EWB is a philosophic concept which tries to be overcome the problems associated with current adhesive systems. The ultimate purpose of this approach is to improve the clinical performance of the contemporary adhesives and extend the longevity of the restorations. This seems ambitious and attractive because the loss of restorations is a great problem in dentistry.

The present study, as previously stated, advocates a hybrid protocol, which comprised the use of ascending ethanol concentrations (70% and 96% only once, for 30 seconds each) during 60 seconds and the use of an experimental hydrophobic primer. This new approach has the aim of being implemented in the everyday clinical practice. Sadek *et al*., 2008 and Ayar *and al*., 2014 showed that full chemical dehydration protocol obtained promising results; nevertheless they are time-consuming, rendering it clinically impracticable. Liu *et al*., 2011 and Khoroushi *et al*., 2014 showed that simplified protocol is technique sensitive and produces lower bond strengths when compared to the previous protocol; nevertheless, this protocol is more user-friendly and has more clinical acceptance. Thus, the new approach proposed in this study represents an attempt to overcome the complications of both protocols, embracing their strengths: promoting strong adhesion through a simple and user-friendly protocol.

After an extensive literature search, the authors outlined their study. Rendering a user-friendly protocol implies that easily available materials should be preferred. Thus, it seems only rational to use commercial adhesive systems. In this study, the phosphoric acid and the composite are not from the same manufacturer as the primer and adhesive. This has the purpose of avoiding possible bias in μTBS values when using all materials from the same manufacturer. The same user-friendly rationale was used to select the ethanol concentrations applied. This is the first study to perform EWB with two ethanol concentrations easily available at a supermarket: 70% and 96%. This contrasts with the full chemical dehydration protocol, which makes use of five different ethanol concentrations. Besides their availability, these two ethanol concentrations have the advantage of being less volatile than higher concentrations, allowing a gradual and effective water displacement (Pashley *et al.*, 2007; Li *et al*, 2012).

In the literature search, it became evident that there was not a consensus about primer application. While some authors applied the commercial primers (Khoroushi *et al*., 2014), application of an experimental hydrophobic primer was advocated by several authors (Sadek *et al*, 2008; Sadek *et al*, 2010a; Sauro *et al*., 2011; Pei *et al*., 2012; Araújo *et al*., 2013). Yet, Mortazavi *et al*., 2012 did not use any primer; instead, the commercial adhesive was applied after the excess ethanol was absorbed. This last protocol seemed interesting because if ethanol removed water effectively, then there seems to be no need to apply a primer composed by hydrophilic monomers. Theoretically, it makes sense to assume that hydrophobic monomers, such as Bis-GMA, present in the adhesives, are able to be coaxed into ethanol-saturated dentin (Nishitani, *et al*., 2006). In order to access the effect of primer application on the EWB technique, the present study compares the application of an experimental hydrophobic primer to the application of no primer in the proposed EWB approach. Then, the two variables chosen for this study are defined.

The experimental hydrophobic primer was obtained by diluting the Adper Scotchbond® MultiPurpose Adhesive with 50 wt% 96% ethanol. The aim of this procedure was to produce a water-free bonding resin with similar composition of the hydrophilic adhesive employed in the water-wet protocol (Sadek *et al*, 2010a). The primer from this adhesive system is water-based and has HEMA and water in its composition. EWB displaces water within dentin, so it seems to be counterproductive to use a water-based primer whenever using a EWB approach.

However, the literature also exhibits a high variability in ethanol application protocols in terms of the required application time. In the quest of a simplified and yet effective protocol, reduced application times should be preferred. As EWB represents an additional step, ethanol must be applied during the minimum time required to be effective Sauro *et al*, 2010 stated that similar results arise when ethanol was applied during 300 seconds (5 minutes) or 60 seconds. Sadek *et al*., 2010a reported that 30 seconds were not enough for complete replacement of water within the dentin by ethanol. Hence, an application time of 60 seconds was chosen.

Contrary to most studies and despite the manufacturer recommendations, the authors increased the curing time of the resin composite. Each composite increment was polymerized during 40 seconds. In fact, Silva, 2008 and Pequeno, 2009 suggested that the 20 seconds recommended by the manufacturer was inferior to the ideal. Perdigão *et*

al., 2006 and Proença *et al*., 2007 obtained less cohesive fractures in composite when the resin composite was cured was cured twice over (4 seconds) the recommended time.

The first null hypothesis was rejected, as immediate bond strength varied, depending on the bonding approach. The results of this study showed that there are not statistically significant differences between the WWB and EWB w/ P. However statistically significant differences were observed between EWB w/o P and the other two bonding approaches, which rejects the second null hypothesis.

Despite the great variability found in protocols, the results of this study are in agreement with previous reports proving that there are not statistically significant differences between WWB and EWB, after 24 hours (Hosaka *et al.*, 2009; Guimarães *et al.*, 2012; Ekambaram *et al.*, 2014; Manso *et al.*, 2014; Yesilyurt *et al.*, 2015).

When solely comparing EWB with WWB, there are no similar studies, since this is the first study that compares 70% and 96% ethanol concentrations. Nevertheless, studies in which commercial etch-and-rinse adhesives, such as Adper Scotchbond® MultiPurpose and Optibond FL, are used were conducted by several authors (Sadek *et al*, 2010a; Mortazavi *et al*., 2012; Pei *et al*., 2012; Khoroushi *et al.*, 2014 and the work of Araújo *et al*., 2012) must be highlighted. Araújo *et al*., 2012 used two ethanol concentrations (50% and 100% for 20 seconds each) and the commercial etch and rinse adhesive Adper Scotchbond MultiPurpose, concluding that there are not statistically significant differences between EWB and the gold standard etch-and-rinse protocols, at 24h. According to the literature, same results could be obtained when booth commercial adhesive systems (Yesilyurt *et al*., 2015; Manso *et al.*, 2014) and experimental primers and adhesives (Hosaka *et al.*, 2009; Ekambaram *et al.*, 2014) were used.

The lack of statistically significant differences between EWB and WWB at 24 hours, despite the adhesive system used, can be purportedly explained by the solubility theory. As stated by Nishitani *et al.*, 2006, when etched dentin was ethanol-saturated, the Hoy's solubility parameter of the collagen matrix was brought closer to those of the ethanol-solvated resins. It is speculated that optimal wetting of collagen fibrils by these solvated resins occurs when the polar surface-free energy components are similar (Barton, 1991). Hence, the significant relationships between resin hydrophilicity and μTBS may be due to the degree of wetting and penetration of acid-etched ethanol-saturated dentin by the resins (Rosales *et al*., 1999; Asmussen & Peutzfeldt, 2005). It is also conjectured

that relatively hydrophobic resins produces higher bond strengths over time (Brackett *et al*., 2005; Ito *et al*., 2005; Nishitani *et al*., 2006. The adhesive used, Adper Schotchbond MultiPurpose Adhesive, is solvent-free and relatively hydrophobic, despite having HEMA in its composition. As described by Tay *et al*., 2007, the sequential steps in EWB technique allow for improved miscibility of the solvated adhesive and the collagen matrix thereby enabling the ethanol-solvated hydrophobic resin blend to infiltrate an ethanol-saturated collagen matrix. It is proposed that diluting this relatively hydrophobic resin represented a crucial step towards the success of this technique. The adhesive polar forces were brought closer to those of the ethanol-saturated dentin, enabling resin monomers infiltration, achieving a relatively homogeneous distribution of hydrophobic resins within the hybrid layer. (Nishitani *et al.*, 2006; Liu *et al*., 2011). EWB technique exhibited statistically similar results to WWB, despite the last being accomplished through the use of a gold standard adhesive. Thus, it was conjectured the adhesive elution was responsible for the success of resin impregnation (Souza Júnior, 2015).

Some authors studied different protocols and achieved different results, registering statistically significant differences when comparing the two bonding approaches at 24 hours (Osorio *et al.*, 2010; Huang *et al.*, 2011; Ayar, 2014). These results may be partially explained due to the technique sensitivity inherent in the EWB bonding philosophy (Osorio *et al*., 2010; Huang *et al*., 2011). Tay *et al*., 2007 have found that any residual water molecule in the collagen fibril network interfered with the infiltration of hydrophobic adhesive monomers, since resin monomers are less soluble in water. Besides that, as proved by Osorio *et al.*, 2010, the etched dentin surfaces exhibited topographical changes depending on the protocol applied. EWB produced smoother surfaces, narrow fibrils and wider interfibrillar spaces, when compared to WWB. These differences may account for variability in bond strengths.

The role of adhesive elution can be further proven since the group where the EWB was applied without a primer, exhibited statistically significant differences when compared to the other two groups. Only three sticks were left for testing, which proves that adhesion was not successfully achieved. Apparently, without the use of a primer, stiff monomer Bis-GMA was not able to fully diffuse into ethanol-saturated dentin. It is suggested that solvating the bonding resin in ethanol was responsible for decreasing the surface activity of the resin, allowing better wetting and penetration (Ounsi *et al*., 2009). It should be noted that although ethanol-saturated dentin might be a better substrate for

adhesive infiltration, the ethanol is highly volatile due to its vapor pressure being greater than that of water, thus compromising the wettability of etched dentin after a short period time (Li *et al*., 2012). Hence, adhesive should be applied within the time window when the dentin matrix is fully saturated with ethanol. On the other hand, Cadenaro *et al*., 2009 and Sauro *et al*., 2001 proposed that Bis-GMA contains hydroxyl groups that can bond with ethanol and that the residual presence of ethanol decreases the initial reaction rate but enhances degree of conversion of resins after 60 seconds exposure. Hence, it is postulated that, without the primer, the ethanol application time must be superior to 60 seconds. These results are not in agreement with the results from the only study in which primer was not applied (Mortazavi *et al*., 2012). In this study, the adhesive applied was OptiBond FL. OptiBond FL does not have the same composition as Adper Schochbond MultiPurpose, which may explain the different outcomes observed.

In the present study, there were no differences in the distribution of fractures across all tested groups. In fact, the most predominant failure pattern was adhesive in WWB, EWB w/ P, EWB w/o P groups (70,9%, 87,3% and 100%, respectively) (Figure 18). This is in accordance with previous studies in literature reporting that, when performing a microtensile bond strength test, more adhesive failures than cohesive failures are expected (Pashley *et al*., 1995; Schreiner *et al*., 1998). In fact, an accurate assessment of the strength of an adhesive material is best determined when the failure occurs within the material itself and does not involve dentin or composite (Sano *et al.*, 1994; Ghassemieh *et al.*, 2008). Sano *et al*., (1994) also states that composite cohesive failures during in vitro tests are not representative of clinical situations, limiting the interpretation of μTBS.

One of the limitations present in this study relates to the scarce literature data available. This limited the experimental study design, since there is little information available to support the study. Besides, as previously stated, there is a plurality of heterogeneous protocols described, which limited the comparisons to the present study.

Another limitation of this study has to do with the lack of long-term water storage. The present study did not evaluate the effects of ageing in the hybrid layers produced by EWB approaches, which is a crucial topic when evaluating the advantages associated with the EWB technique. With this in mind, future studies should analyze the effect of long-term water storage on the *in vitro* performance of a EWB approach.

It is suggested that further investigation should be made. Besides the long term studies, EWB must be study on tertiary dentin, since in a clinical scenario adhesion, adhesion procedures are performed in teeth which were affected by caries. Nanoleakage tests should also be performed.

It is important to state that the *in vitro* nature of this study does not allow direct extrapolation of the results to an *in vivo* situation, so whether the same results would be obtained *in vivo* should be the object of further investigation.

Conclusion:

Within the limitations of the present laboratory study, it may be concluded that, when applied with a primer, the proposed EWB showed similar bonding effectiveness to WWB, after 24 hours. On the other hand, when EWB was applied with no primer, it showed lower bonding effectiveness, when compared to the other two groups.

This has an enormous impact in the adhesive field, since it suggests that contemporary commercial etch and rinse adhesives, considered the gold standard, can be deeply improved. Improving the durability of a restoration affects not only the clinical practice (less failures, less restorations substituted) but also has a deep economic effect on the patients and on the manufacturers.

On the other hand, it can be concluded that some modifications must be made in order to directly bond the ethanol-saturated dentin without using a primer.

Since the EWB approach is relatively new, there is little information in the literature about the in vitro performance of this approach. Future studies should analyze the effect of long-term water storage on the in vitro performance of the proposed protocol.

Besides that, further studies on different substrates, such as tertiary dentin and nanoleakage studies, in association with in vivo tests are needed to assess the long-term clinical behavior of this protocol to support its application.

Clinical significance: Similar bonding effectiveness of the tested ethanol wet bonding approach on the dentin may be obtained if a primer is applied.

APPENDIX I

Materials, Manufacturers, Components and Batch Numbers

Table 9 – *Materials, Manufacturers, Components and Batch Numbers*

Material	Manufacturer	Composition	Batch Number
37,5% Phosphoric Acid Gel Etchant	Kerr Italia S.r.I. Scafati (SA), Italy	Phosporic acid Pigments	Lot 4907232 Exp 07/2016
Adper Scothbond MultiPurpose Primer	3M ESPE Neuss, Germany	HEMA (35–45%) Water (40–50%) Copolymer of acrylic and itaconic acids(10–20%)	Lot N547824 Exp 01/2017
Adper Scothbond MultiPurpose Adhesive	3M ESPE Neuss, Germany	BISGMA (60–70%) HEMA (30–40%) Triphenylantimony (<0.5%)	Lot N655294 Exp 02/2018
Herculite™ XRV Ultra™ Dentine	Kerr Italia S.r.I. Scafati (SA), Italy	ethoxylated Bisphenol A-dimethacrylate; 2,2-ethylenedioxydiethyl Dimethacrylate; 3Methacryloxypropyltrimethoxysilane bisphenol A-glycidyl methacrylate (BIS-GMA)	Lot 5629580 Exp 05/2018
Álcool 70% Vol	Continente,Portugal	Álcool etílico 70% vol 0,25% cetrimida	Lot 15001003 Exp 11/2020
Álcool 96% Vol	Continente Portugal	Álcool etílico 70% vol 0,25% cetrimida	Lot 15001130 Exp 12/2020

APPENDIX II

***Table 10** – Number of sticks obtained in each tooth*

Group	Specimens	Obtained sticks	Debonded sticks	Lost sticks	Tested sticks	Tested sticks by group
1 WWB	1.1	25	9	2	14	111
	1.2	32	6	2	24	
	1.3	34	1	0	33	
	1.4	32	5	2	25	
	1.5	26	8	3	15	
2 EWB w/ P	2.1	36	8	0	28	125
	2.2	28	5	0	23	
	2.3	32	2	0	30	
	2.4	31	4	0	27	
	2.5	23	6	0	17	
3 EWB w/o P	3.1	31	31	0	0	3
	3.2	27	26	0	1	
	3.3	24	22	0	2	
	3.4	47	47	0	0	
	3.5	30	30	0	0	
Total		**459**	**210**	**10**	**239**	**239**

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